Université de Montréal

New strategies for the determination of cyanotoxins using high resolution mass spectrometry

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Cyanobacteria

- **Cyanobacteria**, or blue-green algae, appear naturally, in most cases without toxicity.
- **Intense proliferation** is linked to both human and animal intoxication cases.
- The production of toxic metabolites is the main cause → **cyanotoxins**.
- The production of cyanobacteria, as well as their cyanotoxins, is almost **impossible to predict** precisely.
- Annual cost of harmful blooms> $ 800M in
- the US

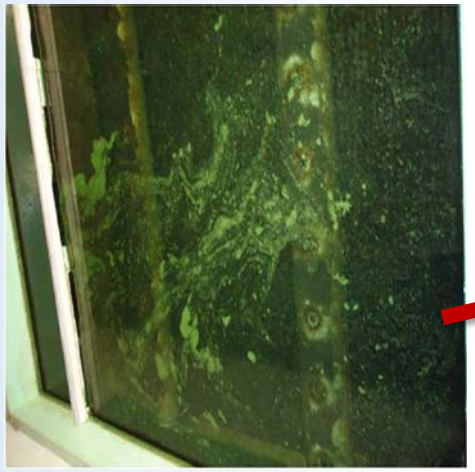
Reporting contaminated water bodies.

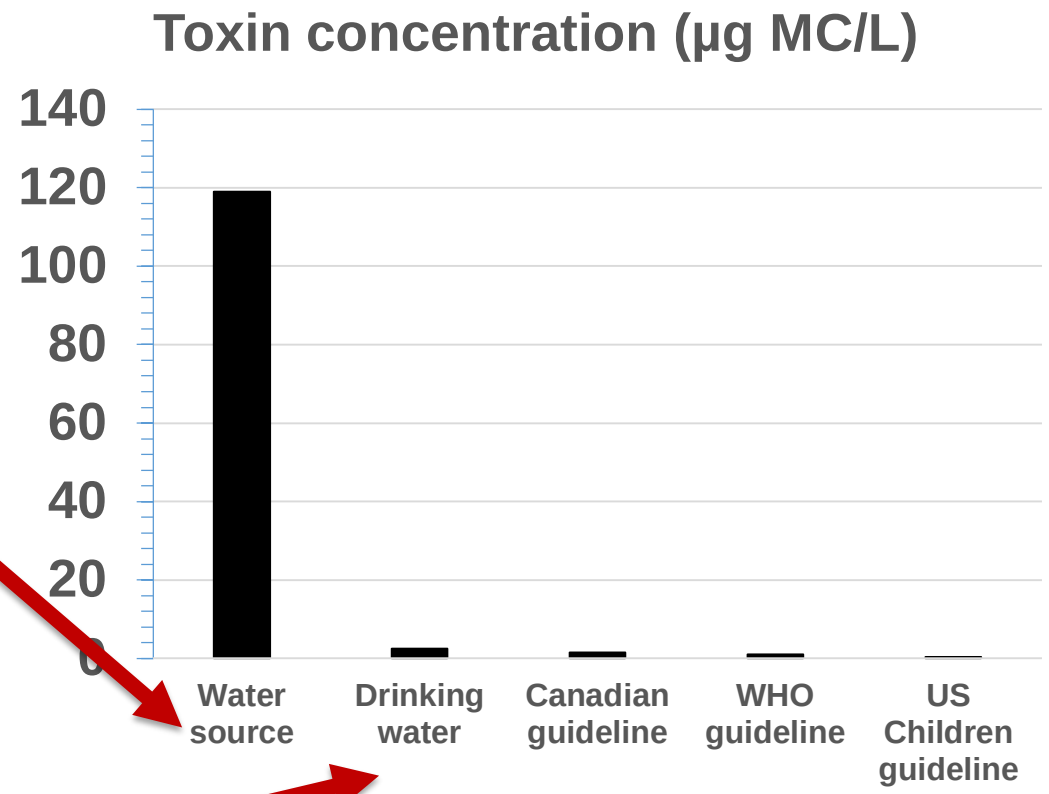
http://saint-mathieu-du-parc.ca/
http://www.ecy.wa.gov/programs/eap/mar_wat/

Widespread algal blooms on Lake Erie

- The growth of blue-green algae is a function of heat and nutrients.
- Agricultural wastes and climate change aggravate the situation.

Drinking water treatment plant





- River water 119 µg MC/L
- Drinking water 2,5 µg MC/L
- Children protection guideline 0.3 µg MC/L (USEPA June 2015)

Problematics

- Cyanotoxins are considered as an emerging threat in aquatic environments but also in water supply systems.
- The development of future analytical methods for cyanotoxins faces many challenges.
- The diversity of toxins and congeners, with different physicochemical proprieties makes it difficult, to date, the ability to detect and quantify the majority of cyanobacterial toxins.
    - various degradation conditions
    - different conditions of storage
    - different extraction / analysis conditions
    - distribution and availability of standards
    - Analytical costs

Determination of β-*N*-methylamino-L-alanine

- The non-proteinogenic amino acid β-*N*-methylamino-L-alanine (**BMAA**) is an excitotoxic neurotoxin.
- BMAA is possibly related to amyotrophic lateral sclerosis/Parkinson's disease complex (ALS/PDC).
- More than **95 % of toxic cyanobacterial** can produce BMAA, suggesting its presence in aquatic environments.
- No regulation and no monitoring are in place in North America.

Cycas circinalis

Cycas circinalis seeds

Roy-Lachapelle, A. *et al*., Analytical Bioanalytical Chemistry, 2015. **407**(18): p. 5487-5501.
Cox P.A., *et al*., P Natl Acad Sci USA, 2005. **102**(27):9734-9734.

Previous work

Several analytical methods have been published for the detection of BMAA, but **few consensuses** have been made on the reported concentrations.

HPLC-FD

- Derivatization with 6-ACQ
- Overestimation → derivatization of other amino acids and small molecules

HPLC-MS & MS/MS

- Derivatization with 6-ACQ for improved selectivity

HILIC-MS/MS

- Simplified method
- High dependency on the chromatographic separation

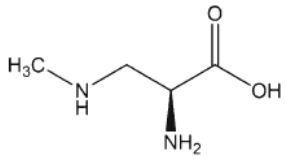
Few methods which include BMAA to other cyanotoxins in a single analysis

Roy-Lachapelle, A. *et al.*, Analytical Bioanalytical Chemistry, 2015. **407**(18): p. 5487-5501.

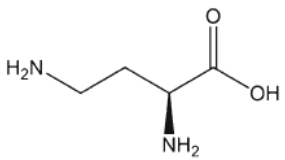
Objectives

The aim of this work is to:

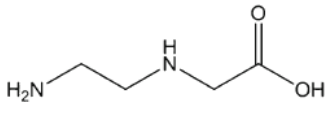
- develop an analytical method for the detection of **BMAA** and two isomers, **DAB** and **AEG,** using **dansyl chloride (DNS) derivatization**.



BMAA



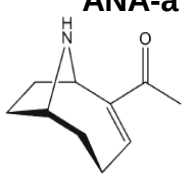
DAB



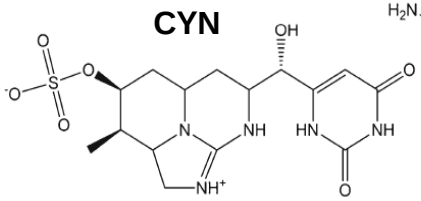
AEG

- include three alkaloid cyanotoxins: **anatoxin-a, cylindrospermopsin and saxitoxin**.

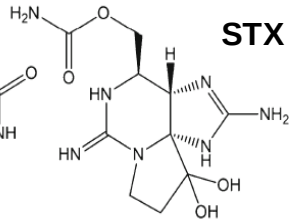
ANA-a

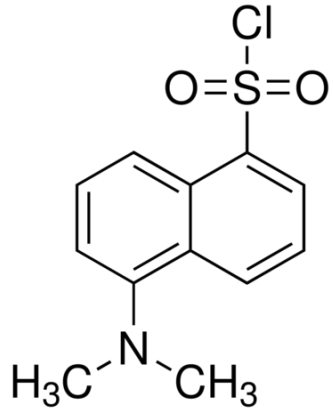


CYN



STX





DNS

- use UHPLC-HESI-HRMS in a fragmentation mode to determine the fragmentation patterns and then suggest their **fragment structures** using Mass Frontier™ 7.0 software.

- apply the method to real field-collected cyanobacterial bloom water samples.

Roy-Lachapelle, A. *et al.*, Analytical Bioanalytical Chemistry, 2015. **407**(18): p. 5487-5501.

Workflow

100 mL lake water

Cell lysis
Freeze/thaw x 3

Internal standard addition

Filtration
Nitrocellulose 0.22 µm

pH to 4 with citric acid

Solid Phase extraction
Cation exchange SPE 200 mg
Conditionning
5 mL MeOH/5 mL H_2O pH 4
Wash
5 mL H_2O pH 4 MeOH (90:10 v/v)
Elution
5 mL MeOH with 3% NH_4OH

Nitrogen drying at 35°C

Reconstitution step
250 µL Borax buffer (0.2 M) + 250 µL DNS (1 mg mL^{-1} in acetone)

Derivatization
60°C, 10 minutes

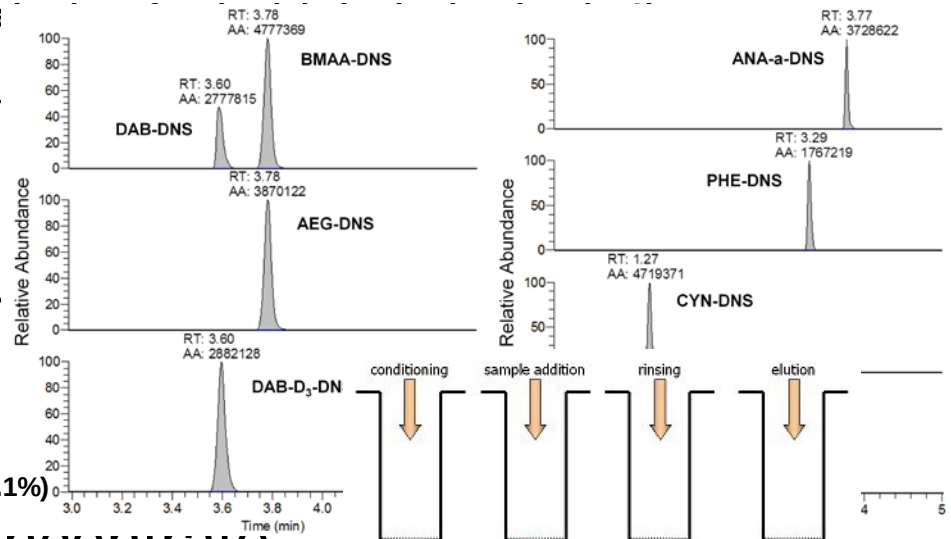
UHPLC-HESI-HRMS analysis

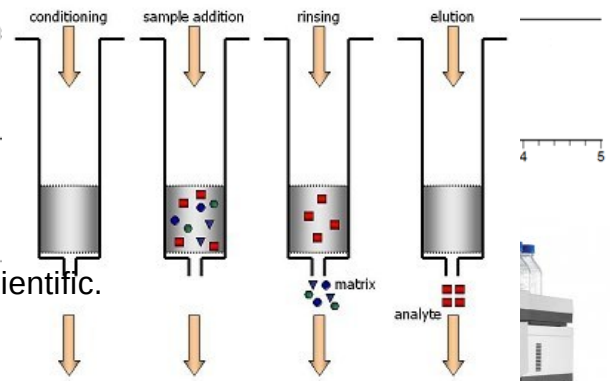
Chromatographic conditions

Eluent gradients

Time (min)	A (%)	B (%)
0.00	70	30
2.00	10	90
4.00	0	100
6.00	0	100
6.01	70	30
10.00	70	30

A: Water + formic acid (0.1%)
B: Acetonitrile + formic acid (0.1%)





Wash

Preconcentrate

Isolate

Thermo Scientific Dionex UltiMate™ 3000 UHPLC.
Q Exactive™ Hybrid Quadrupole-Orbitrap, Thermo Scientific.

- Hypersil GOLD C18 (100 mm, 2,1 mm, 1,9 µm)
- Injection: 25 µL
- Flow: [illegible] $mL\,min^{-1}$
- Temperature: 40 ° C
- HESI-II (+) 3000 V
- PRM: 17,500 FWHM *m/z* 200

Comp
differ
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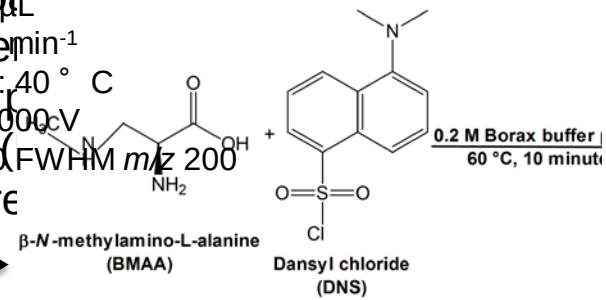
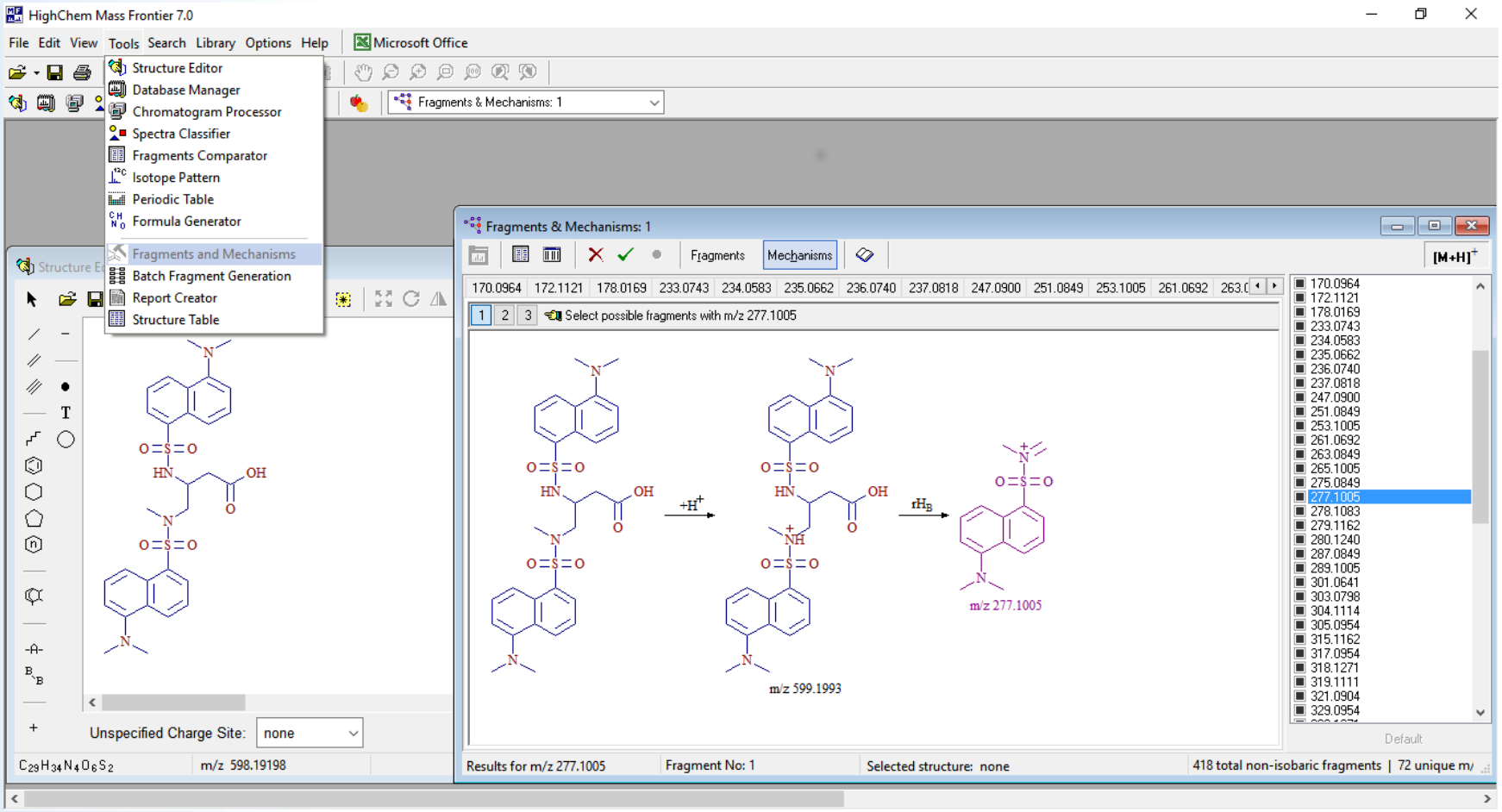


Q Exactive mass spectrometer

Roy-Lachapelle, A. et al., *Analytical Bioanalytical Chemistry*, 2015. **407**(18): p. 5487-5501.
http://www.nationalanalyticalcorp.com/
http://www.thermoscientific.com/en/product/q-exactive-hybrid-quadrupole-orbitrap-mass-spectrometer.html

Fragment identification

Mass Frontier™ 7.0 software



Roy-Lachapelle, A. *et al.*, Analytical Bioanalytical Chemistry, 2015. **407**(18): p. 5487-5501.

Fragments

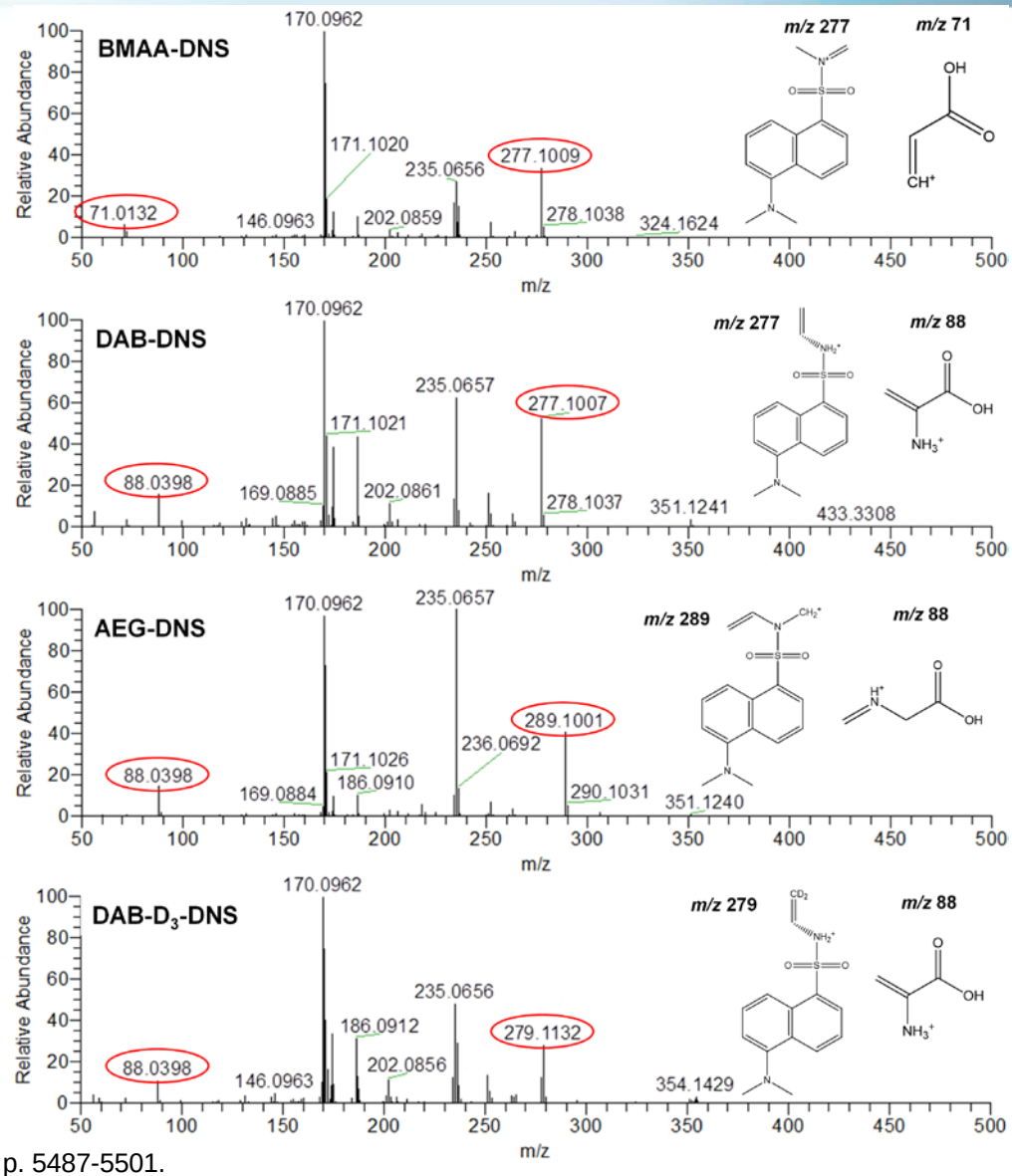
Experimental precursor masses (*m/z*)

- BMAA, DAB and AEG: 585.1836
- ANA-a: 399.1737
- CYN: 649.1744
- SXT: 533.1925
- DAB-D_3: 588.2024

Average mass accuracy < 2 ppm

Due to BMAA and AEG coelution:

Unique fragments are used and their signal ratios are monitored to ensure no false positives are present.



Roy-Lachapelle, A. *et al.*, Analytical Bioanalytical Chemistry, 2015. **407**(18): p. 5487-5501.

Results from bloom water samples

Cyanotoxins detection in lake samples (μg L^{-1}) with relative standard deviation (RSD-%)

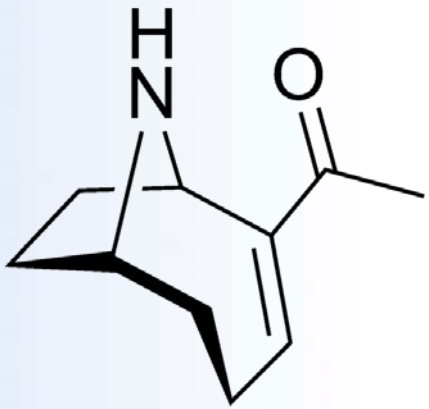
No. Sample	Location	BMAA	DAB	AEG	ANA-a	CYN	SXT
1	Lanaudière	ND	ND	ND	ND	ND	ND
2	Montérégie	ND	**0.01 (7)**	**0.08 (8)**	**0.1 (7)**	ND	ND
3	Montérégie	**0.2 (9)**	ND	ND	ND	ND	ND
4	Montérégie	ND	ND	**0.05 (10)**	ND	ND	ND
5	Estrie	ND	ND	ND	**0.08 (9)**	ND	ND
6	Estrie	**0.03 (8)**	**0.04 (8)**	**0.05 (9)**	**0.02 (7)**	ND	ND
7	Saguenay	ND	**0.009 (10)**	**0.06 (8)**	ND	ND	ND
8	Saguenay	**0.3 (10)**	**0.008 (11)**	**0.009 (11)**	ND	**0.2 (9)**	ND
9	Abitibi-Témiscamingue	ND	ND	ND	**0.2 (6)**	ND	ND
10	Abitibi-Témiscamingue	**0.01 (8)**	ND	ND	ND	ND	ND
11	Abitibi-Témiscamingue	ND	**0.03 (9)**	ND	**0.03 (8)**	**0.1 (11)**	ND
12	Montérégie	ND	ND	**0.01 (5)**	**0.01 (6)**	ND	ND

SPE recoveries: 86 to 103 %
Signal recoveries from matrix effects: 75 to 96 %

Method detection limits (MDL): 0.008 to 0.01 μg L^{-1} → an order of magnitude lower than published methods

Roy-Lachapelle, A. *et al.*, Analytical Bioanalytical Chemistry, 2015. **407**(18): p. 5487-5501.

Determination of anatoxin-a (ANA-a)



pK_a 9.4
log K_{ow} : 1.1

J Vet Diagn Invest 20:89–92 (2008)

Diagnosis of anatoxin-a poisoning in dogs from North America

Birgit Puschner,[1] Brent Hoff, Elizabeth R. Tor

Abstract. Anatoxin-a, a toxin produced by several genera of blue–green algae, is considered a potent neurotoxin. Ingestion of water contaminated with the toxin results in acute neurological signs and often death. This report describes fatal cases of anatoxin-a ingestion in 6 dogs, with confirmation of anatoxin-a exposure by liquid chromatography/tandem mass spectrometry (LC-MS/MS/MS). In 1 outbreak, 3 dogs developed seizures and died within an hour after swimming in a river in California, while the other outbreak involved 3 dogs that died within 1 hour after swimming in a pond in Ontario. Anatoxin-a poisoning is rarely reported in dogs as a cause of acute neurological signs and death. However, increased occurrences of blue–green algae blooms in North America make this neurotoxin an important consideration in the diagnosis of sudden death associated with environmental water exposure. This brief communication reports on the isolation and detection of anatoxin-a from environmental water sources and the stomach contents of North American dogs dying of acute neurotoxicosis. This demonstrates the first documented cases of anatoxin-a poisoning in dogs in North America and the importance of LC-MS/MS/MS in identifying neurotoxins responsible for sudden death in cases of suspected blue-green algae toxicosis; especially those cases showing no gross or histological lesions.

- **Neurotoxin**; Mimics the effect of the neurotransmitter, acetylcholine, resulting in respiratory complications or paralysis.

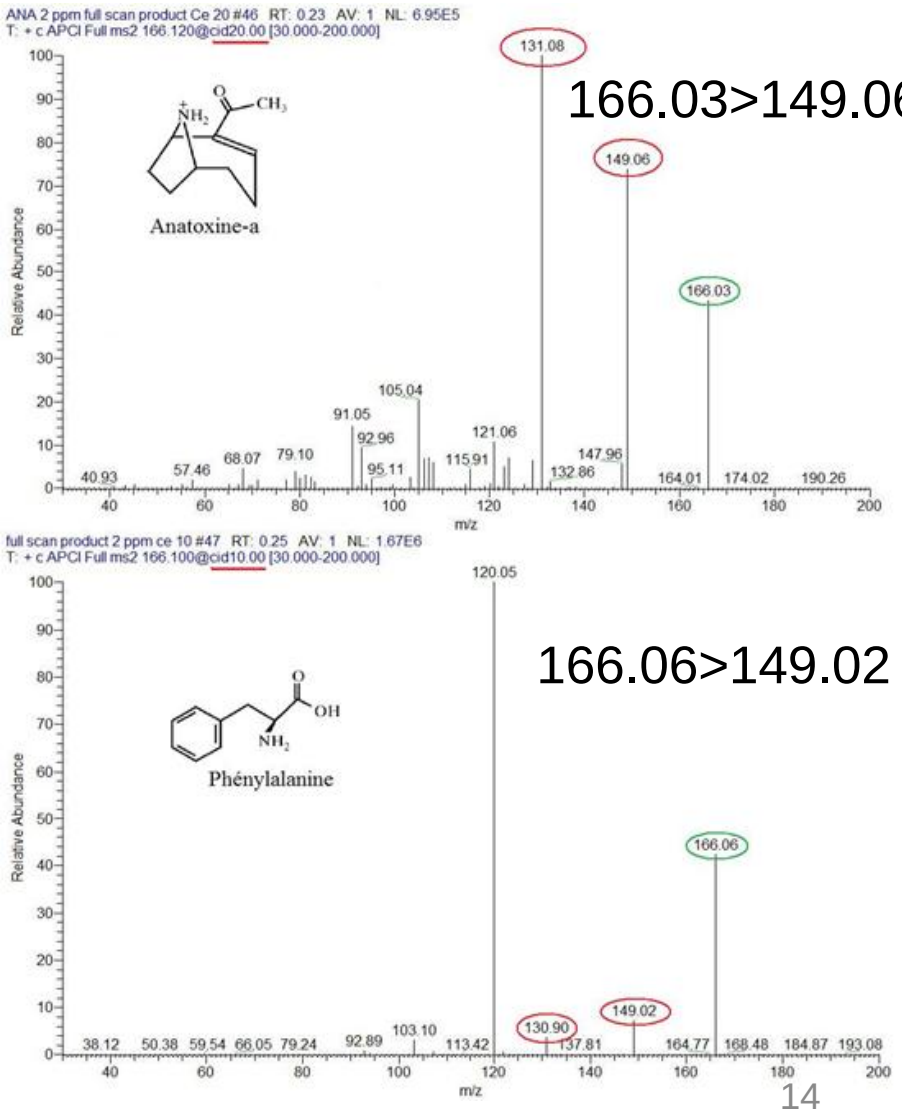
- Many cases of animal mortality (wild and domestic) caused by ANA-a.

- ANA-a is stable under acidic conditions; in presence of UV light or alkalinity → non-toxic transformation products (dihydroanatoxin-a, epoxyanatoxin-a).

Roy-Lachapelle, A. *et al.*, Talanta, 2015. **132**; p. 836-844.
Puschner, B. *et al.*, J Vet Diagn Invest, 2008. **20**; p. 89-92.
Faassen, L. *et al.*, Toxicon, 2012. **60**; p. 378-384.

Analytical challenges

- Spectroscopic detection methods are **avoided** due to the instability of ANA-a in contact with UV light.
- Most of the developed methods are based on **LC-MS/MS**.
- Phenylalanine, an essential amino acid, is an **isobaric interference** of ANA-a using MS/MS detection.
- Some similarities in terms on chromatographic separation, may lead to misindification.

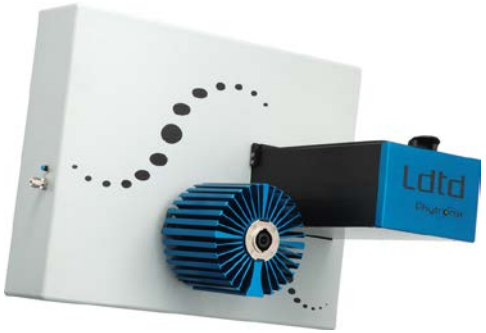


Roy-Lachapelle, A. *et al.*, Talanta, 2015. **132**; p. 836-844.

Objectives

Anatoxin-a analysis using LDTD-APCI-HRMS

- LDTD: Laser Diode Thermal Desorption (Phytronix Technologies)
- instant information of possible contamination
- simple, robust, lower costs, fast (< 10 sec / sample).
- A minimal resolution of **4,500 FWHM** is needed to resolve the mass signals of anatoxin-a and phenylalanine.
- Using PRM (Parallel Reaction monitoring) mode, a resolving power of **17,500 FWHM** (*m/z* 200) with the Q Exactive



LDTD-APCI

Q Exactive

Roy-Lachapelle, A. *et al*., Talanta, 2015. **132**; p. 836-844.
Lemoine, P., Roy-Lachapelle, A., *et al.* Toxicon, 2013, **61**; p. 165–174.
http://ldtd.phytronix.com/
http://www.thermoscientific.com/

Workflow

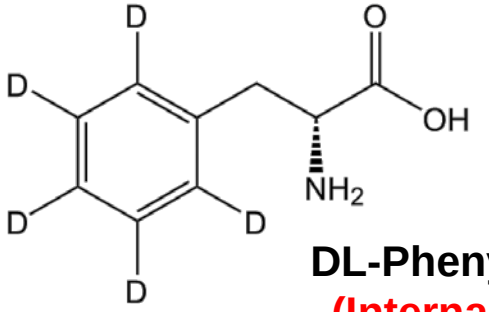
Cell lysis
Freeze/thaw x 3

Internal standard
DL-phenylalanine-D_5

Filtration
0.5 µm nitrocellulose

pH 2 using formic acid (0.1 M)
Dilution with methanol (50:50 v/v)

LDTD-APCI-HRMS analysis (< 10 seconds / sample)



DL-Phenylalanine-D_5
(Internal standard)

Roy-Lachapelle, A. *et al*., Talanta, 2015. **132**; p. 836-844.

ANA-a and phenylalanine resolution

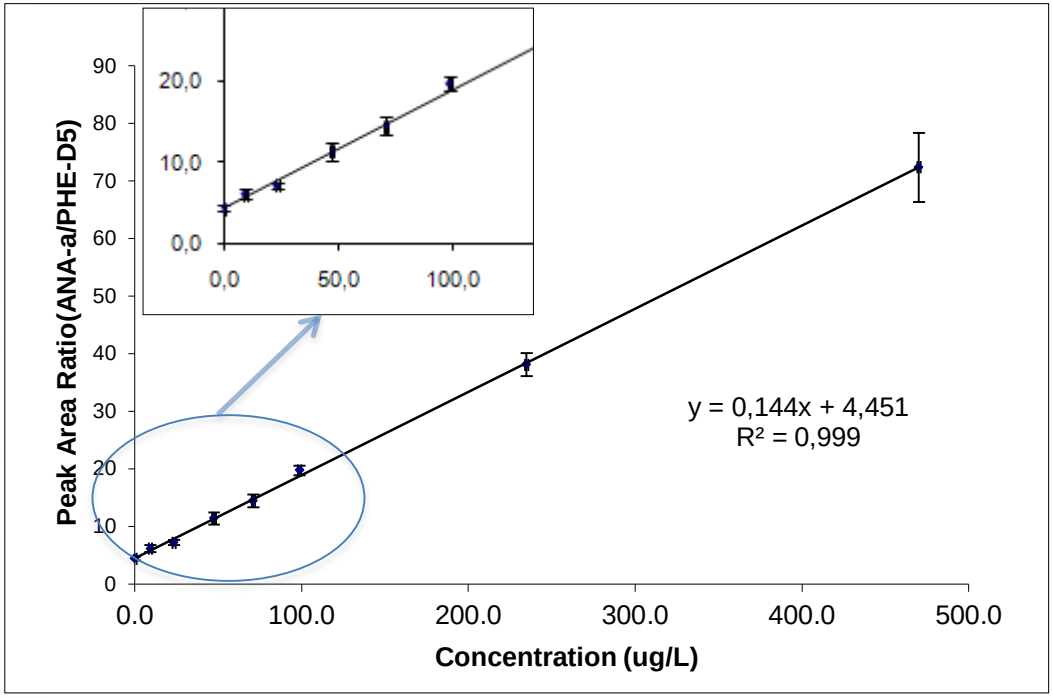
Mass error < 1 ppm

Second most abundant isotopes

Roy-Lachapelle, A. *et al.*, Talanta, 2015. **132**; p. 836-844.

Analysis of ANA-a using the LDTD-APCI-HRMS

Internal calibration by standard addition method (ANA-a/PHE-D5)



Calibration curve showing the linearity of the LDTD experiment

Method detection limit (MDL): 0.2 µg L^{-1}

Method quantification limit (MQL): 0.6 µg L^{-1}

Regulations: **3.7 µg L^{-1}** (Québec) and **6.0 µg L^{-1}** (New Zealand).

Anatoxin-a detection in lake samples

Sample	Location	FS mode detection ($\mu g\ L^{-1}$)	PRM mode detection ($\mu g\ L^{-1}$)	LC-MS/MS* detection ($\mu g\ L^{-1}$)
1	Estrie	2.5 (11)	ND	0.1
2	Estrie	1.1 (9)	ND	0.02
3	Saguenay	ND	ND	0.01
4	Saguenay	ND	ND	0.01
5	Abitibi-Témiscamingue	1.9 (10)	0.21 (7)	0.2
6	Abitibi-Témiscamingue	ND	ND	0.01
7	Abitibi-Témiscamingue	1.6 (12)	ND	0.02
8	Montérégie	ND	ND	0.01

* Quantified by the *Centre d'Expertise en Analyse Environnementale du Québec* (CEAEQ), the analytical services of the *Ministère du Développement Durable, de l'Environnement et de la Lutte contre les Changements Climatiques* (MDDELCC).

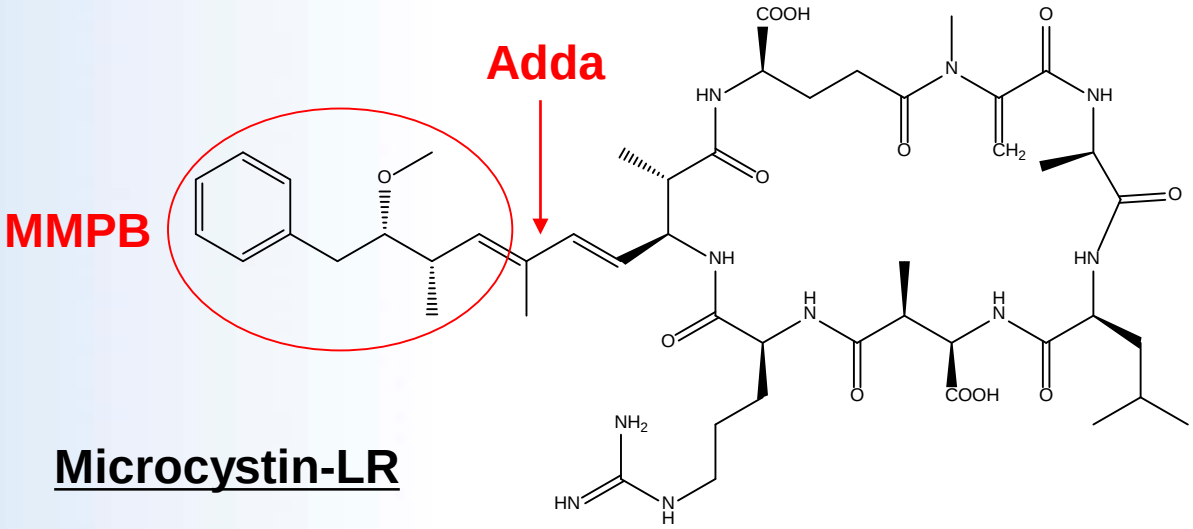
Matrix effects (FS): 98 to 172 %

Matrix effects (PRM): 98 to 102 %

Roy-Lachapelle, A. *et al*., Talanta, 2015. **132**; p. 836-844.

Determination of total microcystins in fish

Microcystin structures (MC)



Microcystin-LR

- More than **100 different microcystins have been identified, but** only a dozen certified standards are available.
- **Regulations in drinking and recreative waters**: 1 µg L^{-1} (WHO) 1.5 µg L^{-1} (Canada).
- In aquatic environments, microcystins can **bioaccumulate**, microcystins may accumulate in food chains and thus be subjected to **biomagnification**.
- This bioaccumulation is due to the formation of irreversible links in the tissues of aquatic species, but is still **largely unknown**.

Roy-Lachapelle A. *et al.*, J. Agric. Food Chem. 2015, **63**; p. 7440−7449.
X. Wu, C. *et al.* Analytical Chimica Acta, 2012. **709**; p. 66-72.

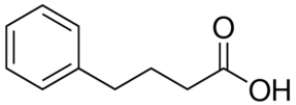
Objectives

Workflow

Lemieux oxidation to extract the MMPB moiety (2-methyl-3-methoxy-4 phenylbutyric acid), common to all microcystins congeners.

1g wet weight - Cell lysis
Freeze/thaw x 3

Internal standard addition
4-Phenylbutyric acid



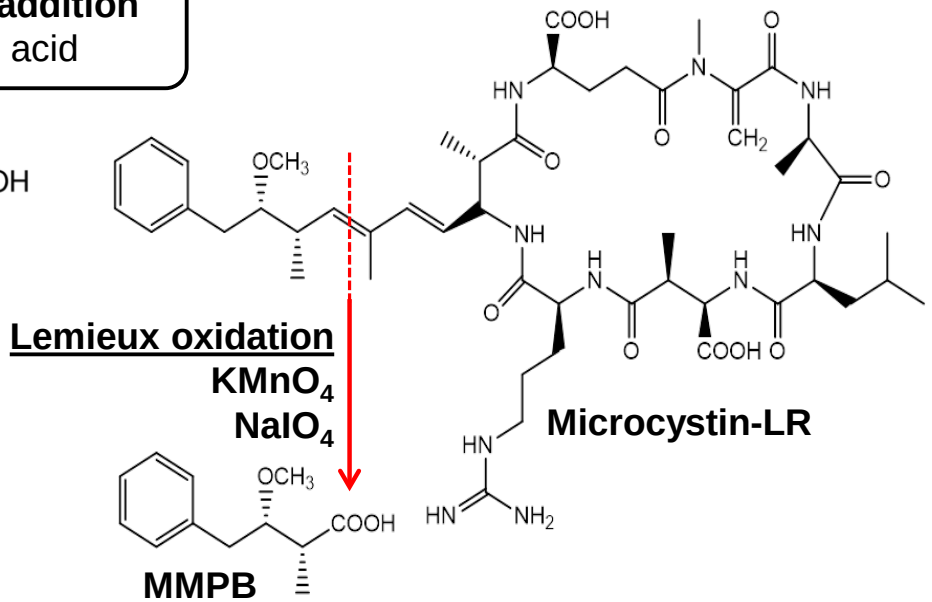
Digestion
5 mL NaOH 50 mM, 2 hours

Oxidation
pH 9 (HCl 100 mM) $KMnO_4$ and $NaIO_4$ 50 mM, 2 hours

Filtration
Nylon filter 0.22 µm

Solid phase extraction:
Styrene divinylbenzene 500 mg
Reconstitution : 500 µL acetonitrile

LDTD-APCI-HRMS analysis



LDTD-APCI

Q Exactive

Roy-Lachapelle A. *et al.*, J. Agric. Food Chem. 2015, **63**; p. 7440−7449.

Results for fish tissue

Comparison of total microcystins found in fish tissue samples using two analytical approaches.

Samples	Lakes	Fish species	Type of tissue	Total MCs via MMPB (µg kg^{-1})[a] (CV - %)	Total MCs with standards (µg kg^{-1})[b]
1	Lac Vert	Rainbow smelt	Viscera	**11.9 (7)**	ND
2	Lac Vert	White sucker	Whole	**8.7 (8)**	ND
3	Lac Vert	White sucker	Whole	**13.2 (6)**	**2.0**
4	Lac Vert	White sucker	Whole	**9.5 (10)**	ND
6	Lac Vert	Brook trout	Muscle	**5.0 (10)**	ND
7	Lac Vert	Brook trout	Muscle	**4.5 (9)**	ND
9	Lac Roxton	Yellow perch	Muscle	**4.0 (10)**	ND
11	Lac Roxton	Yellow perch	Muscle	**3.1 (11)**	ND
14	Lac Roxton	Yellow perch	Muscle	**5.2 (9)**	ND
17	Lac Noir	Yellow perch	Muscle	**4.5 (7)**	ND
18	Lac Noir	Yellow perch	Muscle	**7.3 (11)**	ND
20	Lac Noir	Yellow perch	Muscle	**2.7 (12)**	ND
22	Lac Noir	Walleye	Muscle	**4.4 (8)**	ND
24	Lac Noir	Walleye	Muscle	**2.9 (10)**	ND
27	Lac Noir	Walleye	Muscle	**3.4 (9)**	ND
33	Lac Noir	Brown bullhead	Muscle	**3.8 (9)**	ND
35	Lac Noir	Brown bullhead	Muscle	**5.2 (8)**	ND
38	Lac Noir	Lake whitefish	Muscle	**7.1 (8)**	ND
40	Lac Noir	Lake whitefish	Muscle	**6.2 (9)**	ND
41	Lac Noir	Lake whitefish	Muscle	**5.9 (7)**	ND
44	Lac Noir	Lake whitefish	Muscle	**7.3 (10)**	ND
45	Lac Noir	Lake whitefish	Muscle	**4.5 (11)**	ND
46	Lac Noir	Lake whitefish	Muscle	**3.0 (9)**	ND
49	Lac Noir	Yellow perch	Muscle	**3.7 (11)**	ND

Digestion/oxidation: 70-75%

SPE recuperation : 91-98%

Matrix effects: 90-93%

LDM: 2.7 µg kg^{-1}
LQM: 8.2 µg kg^{-1}

Concentrations between 2.9 and 13.2 µg kg^{-1}

[a]Total microcystins determined via MMPB using LDTD-APCI-HRMS.
[b]Microcystins determined via the summation of all microcystins for which standards allowed detection and quantification (i.e., MC-LA, MC-LR, MC-RR, and MC-YR) using LC-MS/MS.

Roy-Lachapelle A. *et al.*, J. Agric. Food Chem. 2015, **63**; p. 7440−7449.

Results for water samples

Table 3
Comparison of total microcystins analysis of lakes samples using LDTD-APCI-MS/MS and LC–MS/MS.

No. Sample	Location	Date	Total MC via MMPB ($\mu g\,L^{-1}$)[a] (RSD – %)	Total MC with standards ($\mu g\,L^{-1}$)[b]	MC isomer without standards ($\mu g/L$)[c]	Total MC ($\mu g\,L^{-1}$)[d]	Percentage of MC with standards (%)[e]
1	Estrie	20130614	425 (9)	70	340 (RR)[f]	410	16
2	Estrie	20130614	1.0 (5)	0.15	0.5 (RR[f]	0.65	15
3	Saguenay	20130620	5.4 (7)	1.6	3.3 (YR[f]	4.9	30
4	Saguenay	20130620	4.7 (8)	1.8	2.5 (YR[f]	4.3	38
5	Abitibi-Temiscamingue	20130624	ND	ND	ND	ND	–
6	Laurentides	20130731	37.4 (7)	35.4	ND	35.4	95
7	Abitibi-Temiscamingue	20130731	0.9 (8)	ND	ND	ND	–
8	Abitibi-Temiscamingue	20130731	2.7 (7)	0.59	ND	0.59	22
9	Monteregie	20130801	ND	0.1	ND	0.1	–

ND – Not detected.

[a] Total microcystins determined via MMPB using LDTD-APCI-MS/MS.

[b] Microcystins determined via the summation of all microcystins for which standards allowed detection and quantification (i.e., HilR, HtyR, LA, LF, LR, LR (D-Asp3), LW, LY, RR, RR (D-Asp3), WR, YR) using LC–MS/MS.

[c] Microcystin isomer for which identification and concentration could not be certified due to the absence of appropriate standards using LC–MS/MS. Although their characteristics are slightly different from the available standard, there is a very high probability that these are isomers of LC-RR and LC-YR.

[d] Total microcystins determined via the summation of all microcystins for which standards allowed detection and quantification and also for the suspected isomer tentatively identified using LC–MS/MS.

[e] Percentage is calculated as: (MC isomer with standards/total MC via MMPB) × 100.

[f] Suspected isomer of the specified congener.

Determination of cyanotoxins in cyanobacterial dietary supplement samples

Cyanotoxins daily intake from CB dietary supplements samples (µg) according to recommended maximum daily intake. Values in brackets represent the detected cyanotoxins as percentages of the WHO adult TDI guideline of 0.04 µg kg^{-1} body weight for microcystins.

Samples	MCs tot	ANA-a	DH-ANA-a	E-ANA-a	CYN	STX	BMAA
Spirulina	ND	ND	ND	ND	ND	ND	ND
Spirulina	ND	ND	ND	ND	ND	ND	ND
Spirulina	ND	ND	ND	ND	ND	ND	ND
Spirulina	ND	ND	ND	ND	ND	ND	ND
Spirulina	ND	ND	ND	**7.2**	ND	ND	ND
Spirilina	ND	ND	ND	ND	ND	ND	ND
Spirulina	ND	ND	ND	ND	ND	ND	ND
Spirulina	**0.25 (10)**	ND	**0.41**	ND	ND	ND	ND
Spirulina	ND	ND	ND	ND	ND	ND	ND
Spirulina	ND	ND	ND	ND	ND	ND	ND
Spirulina	**2.5 (104)**	ND	ND	**8.1**	ND	ND	ND
Spirulina	ND	ND	ND	ND	ND	ND	ND
Spirulina	**0.6 (25)**	ND	ND	ND	ND	ND	ND
Spirulina	ND	ND	ND	ND	ND	ND	ND
A. Flos-aquae	**16.4 (683)**	ND	**2.2**	**1.8**	ND	ND	**0.08**
A. Flos-aquae	**4.5 (188)**	ND	ND	**0.28**	ND	ND	ND
A. Flos-aquae	**3.3 (138)**	**0.35 (6)**	**5.8**	ND	ND	ND	**0.44**
A. Flos-aquae	**0.8 (33)**	ND	ND	ND	ND	ND	ND

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Analytical automation

Online SPE coupled to UHPLC and HRMS

- Sample extraction automated with the before chromatographic separation.
- Greater analytical capacity.
- Total MCs via MMPB: New method with LOD in water of 0.02 µg ΣMCs/L

14 cyanotoxins:

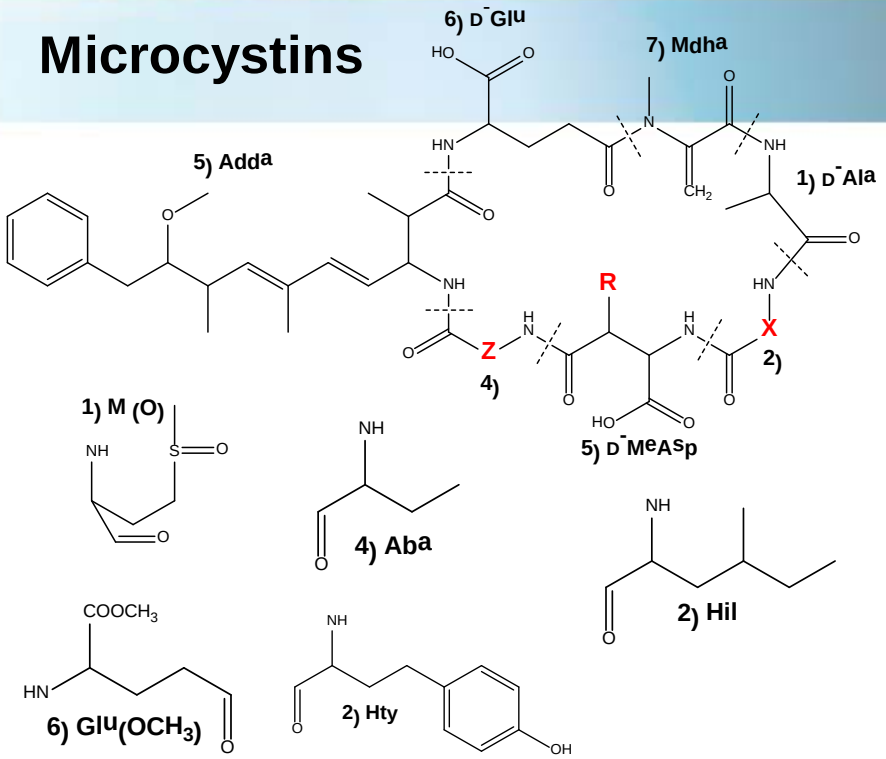
- Using 1 ml of sample with LC-HRMS in 7 min
- HRMS scans: allows to go back on the analyzes to identify known microcystins of which we do not have the standard.
- Inclusion of anatoxin-a transformation products.
- Same strategy for BMAA.

	MLD (µg L^{-1})	MLQ (µg L^{-1})
CYN	0.005	0.02
ANA-a	0.01	0.04
(DAsp^3)MC-RR	0.009	0.03
MC-RR	0.007	0.02
MC-HtyR	0.02	0.05
MC-HilR	0.009	0.05
MC-WR	0.01	0.04
MC-YR	0.01	0.04
MC-LR	0.01	0.04
(DAsp^3)MC-LR	0.006	0.02
MC-LA	0.006	0.02
MC-LY	0.008	0.03
MC-LW	0.01	0.03
MC-LF	0.01	0.04

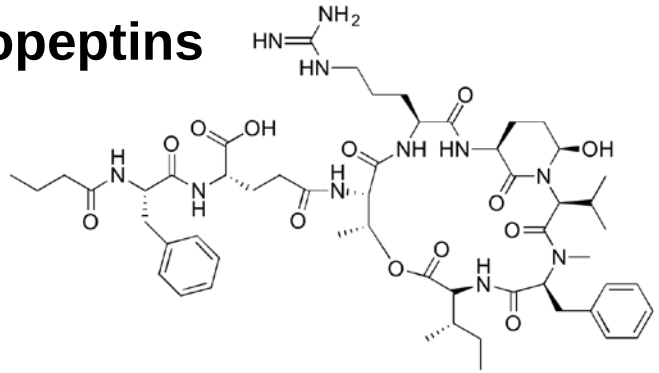
Microcystins congeners

Microcystins		Molecular Formula	Exact Mass	X	Z	R	xLogP$_3$
MC-RR	[Asp^3 Dhb^7] MC-RR	C47H71N13O12	1009.53452	Arg	Arg	H	-0.7
	[Asp^3] MC-RR	**C48H73N13O12**	**1023.55016**	**Arg**	**Arg**	**H**	**-0.4**
	MC-RR	**C49H75N13O12**	**1037.56582**	**Arg**	**Arg**	**CH_3**	**-0.2**
	6(Z)-Adda MC-RR	C49H75N13O12	1037.56582	Arg	Arg	CH_3	-0.2
MC-XR	**MC-YR**	**C52H72N10O13**	**1044.52804**	**Tyr**	**Arg**	**CH_3**	**2.2**
	[Asp^3 Dhb^7] MC-LR	C47H70N10O12	966.51747	Leu	Arg	H	1.5
	[Asp^3] MC-LR	**C48H72N10O12**	**980.53312**	**Leu**	**Arg**	**H**	**2.1**
	MC-LR	**C49H74N10O12**	**994.54877**	**Leu**	**Arg**	**CH_3**	**2.3**
	6(Z)-Adda MC-LR	C49H74N10O12	994.54877	Leu	Arg	CH_3	2.3
	[Asp^3] MC-FR	C51H70N10O12	1014.51747	Phe	Arg	H	
	MC-FR	C52H72N10O12	1028.53312	Phe	Arg	CH_3	2.6
	[Asp^3] MC-WR	C53H71N11O12	1053.52837	Trp	Arg	H	
	MC-WR	**C54H73N11O12**	**1067.54402**	**Trp**	**Arg**	**CH_3**	**2.7**
	[Dhb^7] MC-HilR	C49H74N10O12	994.54877	Hil	Arg	H	2.3
	MC-HilR	**C50H76N10O12**	**1008.56442**	**Hil**	**Arg**	**CH_3**	**2.8**
	[Dhb^7] MC-HtyR	C52H72N10O13	1044.52803	Hty	Arg	H	2.6
	MC-HtyR	**C53H74N10O13**	**1072.55934**	**Hty**	**Arg**	**CH_3**	
MC-RZ	[Asp^3] MC-RA	C45H66N10O12	938.48617	Arg	Ala	H	
	MC-RA	C46H68N10O12	952.50182	Arg	Ala	CH_3	1?
	[Asp^3] MC-Raba	C46H68N10O12	952.50182	Arg	Aba	H	
	MC-Raba	C47H70N10O12	966.51747	Arg	Aba	CH_3	
	MC-RL	C49H74N10O12	994.54877	Arg	Leu	CH_3	
MC-XA	MC-YA	C49H66N7O13	959.46404	Tyr	Ala	CH_3	3.4
	[Asp^3] MC-LA	C45H65N7O12	895.46912	Leu	Ala	H	
	MC-LA	**C46H67N7O12**	**909.48477**	**Leu**	**Ala**	**CH_3**	**3.5**
	[Asp^3] MC-LAba	C46H67N7O12	909.48477	Leu	Aba	H	
	MC-LAba	C47H69N7O12	925.50042	Leu	Aba	CH_3	3.7
	[Asp^3] MC-FA	C48H63N7O12	929.45347	Phe	Ala	H	
	MC-FA	C49H65N7O12	943.46912	Phe	Ala	CH_3	3.4
	MC-FAba	C50H67N7O12	957.48477	Phe	Aba	CH_3	
	[Asp^3] MC-WA	C50H64N8O12	968.46437	Trp	Ala	H	
	MC-WA	C51H66N8O12	982.48002	Trp	Ala	CH_3	3.5
	MC-WAba	C52H68N8O12	996.49567	Trp	Aba	CH_3	
MC-XL	MC-LL	C49H73N7O12	951.53172	Leu	Leu	CH_3	4.8
	MC-FL	C52H71N7O12	985.51607	Phe	Leu	CH_3	
	MC-WL	C54H72N8O12	1024.52697	Trp	Leu	CH_3	4
MC-LZ	**MC-LY**	**C52H71N7O13**	**1001.51099**	**Leu**	**Tyr**	**CH_3**	**4.7**
	MC-LW	**C54H72N8O12**	**1024.52697**	**Leu**	**Trp**	**CH_3**	**5.2**
	MC-LF	**C52H71N7O12**	**985.51607**	**Leu**	**Phe**	**CH_3**	**5.1**
MC-XZ	MC-AW	C51H66N8O12	982.48002	Ala	Trp	CH_3	3.9
	MC-YM	C51H69N7O13S	1019.46741	Tyr	Met	CH_3	4.1
	MC-VF	C51H69N7O12	971.50042	Val	Phe	CH_3	4.7

Microcystins



Micropeptins



Micropeptin 1106, Exact Mass 1105.5808

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